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Condensed Tannins

8. THE ISOLATION AND DISTRIBUTION OF INTERRELATED HEARTWOOD COMPONENTS OF *SCHINOPSIS* SPP.*

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Roux & Maihs (1960a) showed that the distribution of C_{15} 'flavonoid' compounds of the resorcinol series was confined mainly to certain genera of the Leguminosae and two genera of the Anacardiaceae. Roux (1958, 1959) also demonstrated the association between monomeric flavan-3:4-diols and polymeric tannins of the same structural pattern in both families, and later Clark-Lewis & Roux (1959) found that (+)-7:3':4'-trihydroxyflavan-3:4-diol of wattle heartwood (Keppler, 1957) *Acacia mearnsii* (syn. *A. mollissima*) and (-)-7:3':4'-trihydroxyflavan-3:4-diol of quebracho heartwood *Schinopsis quebracho-colorado* (syn. *S. lorentzii*) (Roux, 1958; Freudenberg & Weinges, 1958) were enantiomorphs. Similar distribution of enantiomorphs in the Leguminosae and Anacardiaceae are to be found in the (+)-fustin of *A. mearnsii* (Roux & Paulus, 1960) and (-)-fustin of *Cotinus coggygria* (Freudenberg & Weinges, 1959; Weinges, 1959).

Recent demonstration of the association of the stereochemically interrelated group of flavonoids (+)-7:3':4'-trihydroxyflavan-3:4-diol, (+)-fustin and (-)-fisetinidol in *A. mearnsii* (Roux & Paulus, 1960, 1961), *Acacia decurrens*, *Acacia dealbata* and *Acacia pycnantha* (Roux, Maihs & Paulus, 1961) (Leguminosae) heartwoods now lends interest to a detailed examination of *S. quebracho-colorado* and

Schinopsis balansae (Anacardiaceae) heartwoods for the presence of these and possibly other related substances of opposite rotation.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points were on molecular mixtures of substances (Roux & Maihs, 1960a). Analyses of C, H and methoxyl are by Weiler and Strauss, Oxford. Infrared-absorption curves are by Dr J. R. Nunn, National Chemical Research Laboratory, C.S.I.R., Pretoria. A 2 dm. tube was used for optical rotations.

Isolation of fustin, protocatechuic acid, 2-benzyl-4'-methoxy-2:6:3'-trihydroxycoumaran-3-one, gallic acid and catechin from commercial quebracho extract

Powdered 'ordinary' (i.e. un sulphited) quebracho extract (250 g.) prepared commercially from both *S. balansae* (mainly) and *S. quebracho-colorado* (Howes, 1953) was dissolved in 600 ml. of boiling methanol; the mixture was cooled and 800 ml. of chloroform was slowly added with shaking. The precipitate formed was sucked off and the filtrate evaporated to dryness under vacuum in a rotary evaporator (144.5 g.). The powdered solids were dispersed in 400 ml. of commercial boiling ethyl acetate, and ethanol (75 ml.) was added to effect complete solution. After cooling, 200 ml. of chloroform was added as before. The filtrate gave 40 g. of solids after removal of the precipitate.

Solids (80 g.) obtained by the above method were separated in a Craig machine as described by Roux & Paulus

* Part 7: Roux & Paulus (1961).

(1960, 1961) and after 160 transfers the tubes were examined by paper chromatography on Whatman no. 1 paper.

(\pm)-*Fustin*. Contents of tubes 131–146 were run off separately, the organic phase was separated, and the aqueous phase extracted with ethyl acetate. The combined organic phases were evaporated to small volume (50 ml.) and streaked on sheets (5 ml./sheet) of $22\frac{1}{2}$ in. \times $18\frac{1}{4}$ in. Whatman no. 3 chromatographic paper. The chromatograms were developed by upward migration in 2% acetic acid for 12 hr. Fustin (R_f , 0.42) was located with toluene-*p*-sulphonic acid spray reagent (Roux, 1957b) as a yellow fluorescent band under ultraviolet light. Bands were cut out and eluted with 70% ethanol. A white-pink sludge, containing some crystalline material, separated from the concentrate of the eluates when kept at 0° for 3 days. Brief but rapid heating of the solution caused the sludge to redissolve, leaving fustin crystals. These were sucked off without delay. Recrystallization (charcoal) afforded 80–100 mg. of white crystals m.p. 215–217°. Mixed m.p. with (\pm)-fustin from *Rhus glabra* (Roux & Paulus, 1960) showed no depression; $[\alpha]_D^{25}$ $-9.6 \pm 0.6^\circ$ in acetone-water (1:1, v/v) (c, 0.8). Infrared-absorption curves of these fustins were superimposable over the range 2.5–15 μ . Fustin (70 mg.) from commercial quebracho extract was acetylated as for (+)-fustin (Roux & Paulus, 1960), giving 80 mg. of tetra-acetylfustin, m.p. 149°, $[\alpha]_D^{25}$ $-7.2 \pm 0.2^\circ$ in tetrachloroethane (c, 1.0). Mixed m.p. with tetra-acetyl-(\pm)-fustin showed no depression.

Compared with the pure optical isomers (–)-fustin, $[\alpha]_D$ -26° (Weinges, 1959), and (+)-fustin, $[\alpha]_D$ $+28.3^\circ$, and their tetra-acetyl derivatives, $[\alpha]_D$ -25.2° and $+24.4^\circ$ respectively (Roux & Paulus, 1960), the rotation of (\pm)-fustin and its acetate from quebracho apparently consists of a 2:1 proportion of (–)- and (+)-enantiomers.

Protocatechuic acid. Substance A (R_f , 0.50), running in approximately the same position as fisetinidol relative to fustin (Roux & Paulus, 1961), was obtained (30 mg.) from tubes 116–130 by the procedure outlined above. After recrystallization its m.p. (197–199°) and crystal shape (needles) differed from those of fisetinidol (m.p. 211–214°, rhombic), and a mixed m.p. with authentic protocatechuic acid showed no depression. Infrared-absorption curves of substance A and protocatechuic acid were identical over the range 2.5–15 μ . Substance A and protocatechuic acid gave identical colorations with ferric alum (olive green), diazotized *p*-nitroaniline (Swain, 1953) (canary yellow) and when oversprayed with sodium carbonate (lilac), and substance A was also acidic.

2-Benzyl-4'-methoxy-2:6:3'-trihydroxycoumaran-3-one. Component B was eluted from bands from the same sheets from which protocatechuic acid and (\pm)-fustin were obtained. The bands were located with ammoniacal silver nitrate spray and eluted with 70% ethanol as before. White crystals (200 mg.) separated easily from the eluates after concentration, m.p. 194°. Recrystallization from water (charcoal) increased the m.p. to 196°; $[\alpha]_D^{25}$ $-1.2 \pm 0.2^\circ$ in acetone-water (1:1, v/v) (c, 1.0) (Found: C, 64.7; H, 5.2; –OMe, 8.9. Calc. for $C_{16}H_{14}O_6$: C, 63.6; H, 4.7; –OMe, 10.3%). These values agree fairly closely with those obtained by King (1957) for a compound running to approximately the same position as component B (cf. King & White, 1957a), and to which he has assigned the structure 2-benzyl-4'-methoxy-2:6:3'-trihydroxycoumaran-3-one.

The infrared-absorption spectrum (KBr) of component B has strong absorption bands at 3250 cm^{-1} (hydroxyl), at 1690 cm^{-1} (non-chelated aryl ketone), at 1615, 1590 and 1508 cm^{-1} (C=C aromatic), 1270 cm^{-1} (C=O aromatic),

1120 cm^{-1} (aryl-O-C) and 1038 cm^{-1} (OMe) (see

Stamm, Schmid & Buchi, 1958), which appear to confirm the structure proposed by King (1957).

(+)-*Catechin*. The solids (8 g.) from tubes 66–71 were separated by chromatography on 20 sheets of Whatman no. 3 paper (400 mg./sheet) with 2% (v/v) acetic acid as irrigant. Compound C was located by spraying a test strip with bisdiazotized benzidine (Lindstedt, 1950). Two-dimensional chromatography showed that gallic acid accompanies compound C in the eluates. The solids (1.6 g.) were separated on eight sheets of Whatman no. 3 paper, previously washed in water, by using butan-1-ol-acetic acid-water (6:1:2, by vol.) (Nordström & Swain, 1953) as irrigant. Bands corresponding to catechin and gallic acid were eluted. The catechin (0.1 g.), although apparently pure, did not crystallize but gave the identical colour reactions and ran to the same position as (+)-catechin on a two-way paper chromatogram with butan-2-ol saturated with water, followed by 2% acetic acid, which permits the separation of all stereoisomers of catechin.

(–)-7:3':4'-Trihydroxyflavan-3:4-diol, which is present in the same tubes as (+)-catechin (see below) when peripheral heartwood components are subjected to Craig separations, was entirely absent from the commercial extract.

Gallic acid. Gallic acid (200 mg.), m.p. and mixed m.p. with authentic specimen 253°, was obtained from the (+)-catechin fraction (Craig tubes 66–81), although most of the gallic acid is distributed in tubes 81–101.

(\pm)-*Fustin from the heartwood of Schinopsis quebracho-colorado (syn. Schinopsis lorentzii)*

Wood from the outer perimeter of the heartwood of a 42-year-old specimen was removed by drilling with a $\frac{3}{8}$ in. drill. The drillings (500 g.) were extracted three times with methanol in the cold (24 hr./extraction), and the combined extracts were evaporated to dryness in a rotary evaporator. The solids (120 g.) were easily soluble in the lower phase (400 ml.) of water–butan-1-ol–light petroleum (b.p. 70–90°) (5:3:2). The solution was introduced into the first eight tubes of a 50 ml. underphase Craig machine, and the mixture separated by countercurrent partition separation as described by Roux & Paulus (1960, 1961).

Fisetin (146–160), fustin (116–141), 2-benzylcoumaranones (King, 1957; King & White, 1957a, b) (96–121), gallic acid (55–101), (–)-7:3':4'-trihydroxyflavan-3:4-diol (40–80) and (+)-catechin (50–80) were present in the tube numbers indicated. (+)-Catechin, (–)-7:3':4'-trihydroxyflavan-3:4-diol and gallic acid are the main polyphenolic components and have been isolated from this wood (Roux & Evelyn, 1960). Fustin (200 mg.) was separated from tubes 116–141 by the methods described above, but tannins did not interfere with their crystallization; m.p. 220°. $[\alpha]_D^{25}$ $-3.8 \pm 0.6^\circ$ in acetone-water (1:1, v/v) (c, 0.8). Tetra-acetyl derivative, m.p. 150–151°. $[\alpha]_D^{25}$ $+0.9 \pm 0.3^\circ$ in tetrachloroethane (c, 1.1). Mixed m.p. with tetra-acetyl-(\pm)-fustin showed no depression.

Table 1. *Radial and vertical distribution of the heartwood components of Schinopsis quebracho-colorado (42 years old)*

Benzylcoumaranones separate in the solvent systems used, but could not be estimated accurately as 2-benzyl-2:6:3':4'-tetrahydroxyflavan-3-one was not isolated, and the isolated 4'-methoxyl derivative showed slow reducing action. Their relative concentrations were therefore indicated by + and × respectively. In sample 2 (ground level) the 4'-methoxyl derivative appears to be present over the range 1-3%.

Average height above ground of cross-section (in.)	Sampling position	(-)-7:3':4'-Trihydroxyflavan-3:4-diol (%)	(+)-Catechin (%)	(±)-Fustin (%)	Fisetin (%)	Benzylcoumaranones	Gallic acid (%)
<i>Sample 1 (Roux & Evelyn, 1960)</i>							
206.5	1	6.0	3.7	0.0	0.0	-	1.5
104	02	9.5	3.8	1.4	0.0	-	1.7
	1	4.5	4.3	1.6	0.0	+	1.3
	2	8.0	3.3	1.0	0.1	+	1.2
37	04	5.7	3.6	0.0	0.0	-	1.6
	03	7.3	2.5	1.0	1.2	+	1.7
	02	5.5	1.6	0.8	1.3	++	1.4
	1	2.6	1.2	1.1	1.1	++	0.7
	2	4.3	1.6	0.6	1.0	++ × ×	1.5
	3	5.5	3.0	0.8	0.6	++ × ×	1.3
	4	4.6	3.6	0.2	0.0	-	2.5
8	05	5.7	2.6	1.1	0.4	+ ×	2.2
	04	2.3	1.7	1.3	0.8	++ ×	1.6
	03	1.1	1.7	0.8	1.1	- ×	2.2
	02	2.6	1.4	1.3	1.8	++ ×	1.9
	1	4.2	1.9	1.1	2.0	++ ×	1.9
	2	1.3	2.2	1.4	1.3	+ ×	2.2
	3	6.4	3.6	1.2	0.4	-	3.3
<i>Sample 2 (Roux & Evelyn, 1958)</i>							
Ground level	05	3.2	1.9	0.3	2.5	+++	2.8
	04	3.0	1.1	0.3	3.0	++ (×)	2.5
	03	3.0	1.3	1.4	3.0	+ × × ×	1.9
	02	0.8	0.7	1.5	2.2	(+) × × ×	1.3
	1	0.2	0.6	1.7	2.2	(+) × × ×	1.5
	2	1.2	0.6	1.2	2.3	- × ×	1.7
	3	2.7	1.3	1.2	2.0	(+) × ×	2.0
	4	4.2	1.8	1.4	3.0	++ × ×	2.5
	5	1.9	1.4	0.5	1.6	(+)	2.8

(-)-7:3':4'-Tetrahydroxyflavan-3:4-diol (900 mg.) was isolated from tubes (40-80) by the same methods as those employed for the (±)-fustin.

Absence of (+)-fisetinidol. (-)-7:3':4'-Tetrahydroxyflavan-3:4-diol (500 mg.) was hydrogenated as described by Weinges (1958) to give 300 mg. of (+)-fisetinidol, m.p. 211-214°. Chromatographic comparisons showed that (+)-fisetinidol was completely absent from those Craig tubes (110-140) in which its presence was expected.

(±)-Fustin from the heartwood of
Schinopsis balansae

Three small specimens of the heartwood from different regions of the Argentine were combined (500 g.), and the methanol extractives of the drillings and powdered wood (130 g.) were separated in the Craig machine. Fustin, m.p. 220° (100 mg.), was isolated as before, and converted into the tetra-acetyl derivative m.p. 146-147°; $[\alpha]_D^{23} - 6.1 \pm 0.4^\circ$ in tetrachloroethane (c, 0.8). Compared with the specific

rotation of the pure tetra-acetyl(-)-fustin (24.4°), this value shows that the racemate contains approx. 62.5% of (-)-fustin. (+)-Fisetinidol was also absent in the heartwood components of *S. balansae*. Traces of protocatechuic acid were present.

Radial and vertical distribution of (-)-7:3':4'-trihydroxyflavan-3:4-diol, (+)-catechin, gallic acid, fustin, fisetin and 2-benzylcoumaranones in Schinopsis quebracho-colorado

Radial samples of cross-sections of a 42-year-old specimen of *S. quebracho-colorado*, obtained by drilling, have been examined for average molecular weight of tannins, total leuco-fisetinidin content and tannin/non-tannin ratio (Roux & Evelyn, 1958) and also vertically at different heights (Roux & Evelyn, 1960). By the methods of Roux & Maihs (1960a) the radial and vertical distributions of the above compounds were estimated by chromatographic and densitometric methods with the identical specimens

examined previously (Roux & Evelyn, 1958, 1960). Ammoniacal silver nitrate spray reagent was used for (-)-7:3':4'-trihydroxyflavan-3:4-diol and bisdiazotized benzidine (Lindstedt, 1950) for (+)-catechin. Gallic acid was estimated with bisdiazotized benzidine, but, owing to overlap of components, aq. 3% ferric alum reagent, freshly made, was used for visual confirmation of the concentration. Fisetin, fustin and the 2-benzylcoumaranones were estimated from sheets where the ammoniacal silver nitrate spray had been used. In some samples, where slight overlap of other components occurred, visual estimation of fisetin and fustin provided a check on the accuracy of the densitometric method. Precise details of the spray reagents and methods are described by Roux & Maihs (1960b). The distribution of components is shown in Table 1.

DISCUSSION

King & White (1957a, b) have already drawn attention to a biogenetic metabolic relation between the structurally related compounds 2-benzyl-2:6:3':4'-tetrahydroxycoumaran-3-one, its 4'-methyl ether, fisetin, 4'-methoxyfisetin, fustin and leuco-fisetinidin, all of which occur in quebracho heartwoods. The benzylcoumaranones (King, 1957) and the fisetins (Perkin & Gunnell, 1896; Kirby & White, 1955) and (-)-7:3':4'-trihydroxyflavan-3:4-diol (Roux, 1958; Freudenberg & Weinges, 1958; Roux & Evelyn, 1958) have been isolated and chromatographic evidence has been given for the presence of fustin (King & White, 1957a).

Fustin has now been isolated in low yield (0.02%) from commercial quebracho extract and from the heartwoods of *S. balansae* and *S. quebracho-colorado*. The high melting points of these fustin acetates (146–151°) compared with the pure optical isomers (116–118°) showed that fustin was present as a racemate in each instance (Roux & Paulus, 1960). A predominance (66%) of (-)-fustin was, however, present in the commercial extract and in samples of *S. balansae* examined. In view of the association of related 2:3-dihydroflavonols and flavan-3:4-diols of the same rotation in a number of woods (Weinges, 1958; Freudenberg & Weinges, 1959; Weinges, 1959; Roux & Paulus, 1960) the association of (-)-fustin and (-)-7:3':4'-trihydroxyflavan-3:4-diol in the heartwoods of *Schinopsis* spp. was expected. This appears at least partly valid for the commercial extract derived mainly from the heartwood of *S. balansae* (Howes, 1953).

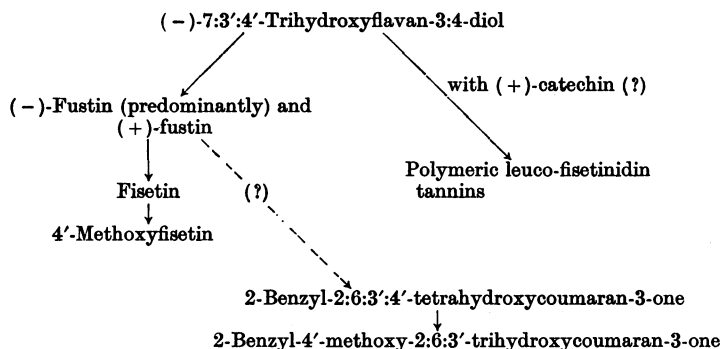
(+)-Fisetinidol, a catechin which is related chemically and stereochemically to (-)-7:3':4'-trihydroxyflavan-3:4-diol (Weinges, 1958) was absent from *S. balansae*, *S. quebracho-colorado* and the commercial extract. Protocatechuic acid was isolated from the commercial extract and is also present in traces in *S. balansae* heartwood.

Of the benzylcoumaranones (King & White, 1957a; King, 1957) the 4'-methoxyl derivative of

2:6:3':4'-tetrahydroxycoumaran-3-one apparently predominates in the commercial extract and may readily be separated by Craig countercurrent separation followed by adsorptive separation on cellulose. The optical inactivity (King, 1957) of the substance was confirmed, and an interpretation of its infrared absorption curve apparently supports the structure assigned by King (1957).

Estimation of the radial and vertical distribution of (-)-7:3':4'-trihydroxyflavan-3:4-diol and (+)-catechin in *S. quebracho-colorado* confirms the visual observation (King & White, 1957a; Roux, 1958; Roux & Evelyn, 1958) of their decrease with increasing age of the heartwood, and provides an accurate assessment of their distribution in the heartwood for the first time. Changes which occur in the concentration of these substances may now be linked directly with the changes in tannin/non-tannin ratio, average molecular weight and total leuco-fisetinidin content observed for the same specimens (Roux & Evelyn, 1958, 1960). (-)-7:3':4'-Trihydroxyflavan-3:4-diol and (+)-catechin are present in high concentration in the first heartwood to be laid down in the growing tree (height 206.5 in., Table 1) and in the peripheral heartwood (heights 37, 8 and 2 in., Table 1) in the mature portion of the tree. These compounds increase in concentration slightly further inwards (at 37, 8 and 2 in. levels) in the mature heartwood and where the young heartwood widens (104 in. level), to reach a maximum, and then decline in concentration toward the centre of the heartwood (104, 37 and 2 in. levels). The even gradation of (-)-7:3':4'-trihydroxyflavan-3:4-diol is sometimes upset (8 in. level), possibly owing to some growth irregularity. Gallic acid concentration also declines slightly towards the centre of the heartwood. In trees of advanced age (over 100 years old) the peripheral heartwood containing the (-)-7:3':4'-trihydroxyflavan-3:4-diol and (+)-catechin is very narrow, and the central heartwood from which the commercial extract is derived (Howes, 1953) contains no (-)-7:3':4'-trihydroxyflavan-3:4-diol, a trace of (+)-catechin, but relatively high concentrations of gallic acid.

The progressive decrease of (-)-7:3':4'-trihydroxyflavan-3:4-diol with age of the heartwood appears to be associated with increases in the proportions of (±)-fustin, fisetin and related benzylcoumaranones (Table 1). This, together with the predominance of (-)-fustin in *S. balansae*, suggests that in addition to the formation of leuco-fisetinidin tannins (Roux, 1957a, b) interconversions may also occur according to Scheme 1. The origin of the 2-benzyl-2-hydroxycoumarones is not obvious, but they have been formed by the action of alkali on dihydroflavonols (Gripenberg, 1957). The radial decline of (+)-catechin possibly indicates that this substance



Scheme 1

may also be involved in tannin formation as suggested by King & White (1957*a, b*).

These radial changes in the heartwood of *Schinopsis* spp. are accompanied by a parallel increase in molecular weight and tannin/non-tannin ratio (Roux & Evelyn, 1958, 1960), as well as by an apparent pattern of interconversion and condensation of a parent flavan-3:4-diol similar in some respects to that visualized for the heartwood of *A. mearnsii* (Roux & Paulus, 1961).

SUMMARY

1. (\pm)-Fustin has been isolated from the heartwoods of *Schinopsis balansae*, *Schinopsis quebracho-colorado* and from commercial quebracho extract. (-)-Fustin predominates (66%) in the extract of *S. balansae* and in the commercial extract.

2. Other known and some related components have also been isolated by means of Craig counter-current separations followed by adsorption separation on cellulose.

3. The radial and vertical distribution of (-)-7:3':4'-trihydroxyflavan-3:4-diol, (+)-catechin, gallic acid, (\pm)-fustin, fisetin and 2-benzyl-2-hydroxycoumaran-3-ones in the heartwood of *S. quebracho-colorado* has been estimated.

4. The possible role these compounds play in the biogenesis of tannins and the interconversions of the compounds related to (-)-7:3':4'-trihydroxyflavan-3:4-diol are discussed.

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